Published in final edited form as:

Heart Rhythm. 2011 May ; 8(5): 704–710. doi:10.1016/j.hrthm.2011.01.003.

A Common Variant in the Beta-2 Adrenergic Receptor and Risk of Sudden Cardiac Death

Michael C Gavin, MD, Christopher Newton-Cheh, MD, MPH, John Michael Gaziano, MD, MPH, Nancy R. Cook, ScD, Martin VanDenburgh, and Christine M. Albert, MD, MPH
Center for Human Genetic Research and Cardiovascular Research Center, Massachusetts General Hospital, (C.N.-C.) Boston, MA; Broad Institute of Harvard and MIT (C.N.-C.), Cambridge, MA; Massachusetts Veterans Epidemiology, Research and Information Center and Geriatric Research Education and Clinical Center (J.M.G), Boston VA Healthcare System; Center for Arrhythmia Prevention (C.M.A), Division of Aging (J.M.G), Division of Preventive Medicine (J.M.G, N.R.C, M.V, C.M.A), Cardiovascular Division (C.M.A), Brigham and Women's Hospital, Harvard Medical School, Boston, MA; Cardiovascular Division (M.C.G.), Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; and Department of Epidemiology (N.R.C), Harvard School of Public Health, Boston MA

Abstract

Background—Homozygosity for a common non-synonymous single nucleotide polymorphism (Gln27Glu) in the beta-2 adrenergic receptor gene (*ADRB2*) has been inconsistently associated with sudden cardiac death (SCD) in individual studies of small sample size.

Objectives—To examine the association between the Gln27Glu polymorphism and SCD in a large combined sample of SCD cases.

Methods—Nested case-control analysis among individuals of Caucasian ancestry enrolled in six prospective cohort studies. Genotypes for the Gln27Glu variant were determined for 492 cases of SCD and 1388 controls matched on age, sex, cohort, follow-up time, and history of cardiovascular disease (CVD) and at the time of the blood draw. Individual studies were combined with conditional logistic regression with fixed effects meta-analysis assuming a recessive model.

Results—Homozygosity for the Gln27 allele conferred a non-significant elevation of the age-adjusted odds ratio (OR=1.22; 95% CI, 0.98-1.53; $P=0.08$) for SCD, which became marginally significant after controlling for multiple cardiac risk factors (OR=1.30, 95% CI: 1.01-1.67; $P=0.046$). In secondary analyses using controls additionally matched for the development of nonfatal CVD after the blood draw, results were attenuated (OR=1.19, 95%CI: 0.92-1.52; $P=0.19$). When the results of the primary analysis were combined in meta-analysis with published reports, a significant association between *ADRB2* genotype and SCD emerged (OR=1.35, 95% CI: 1.15-1.60; $P=0.0003$).

Conclusions—These data from a large prospective case-control series, when combined with published studies, provide further evidence for an association between *ADRB2* genotype and SCD. The mechanism is unknown, but appears to be partly mediated by development of CVD.

Correspondence to: Christine M. Albert, MD, MPH, Center for Arrhythmia Prevention, Division of Preventive Medicine, Cardiovascular Division, Brigham and Women's Hospital, 900 Commonwealth Avenue East, Boston, MA 02115-1204, Tel: (617)278-0807, Fax: (617)277-0198, calbert@partners.org.

Conflicts of Interest: None

Keywords

Sudden Cardiac Death; Genetic Epidemiology; Sympathetic Nervous System; Beta-2 Adrenergic Receptor

INTRODUCTION

There are an estimated 250,000-400,000 sudden cardiac deaths (SCD) annually in the United States^{1, 2} accounting for over 50% of all coronary heart disease^{3, 4}. The majority of these deaths occur as the first manifestation of heart disease^{4, 5}; therefore, reductions in SCD incidence will require improved risk stratification and preventive strategies within the general population. Since there is a heritable component to SCD risk within populations⁶⁻⁸, genetic markers may allow us to better identify individuals within the general population who are at an elevated risk for SCD when utilized in combination with other risk markers. In addition, improved understanding of the genetic determinants of SCD within populations may illuminate biologic pathways involved in the genesis of lethal ventricular arrhythmias, which could ultimately lead to new therapeutic approaches for SCD prevention.

Neurohormonal activation through the adrenergic nervous system is recognized as an important pathway modulating vulnerability to ventricular arrhythmias in diverse disease states^{9, 10}. One of the first common genetic variants reported to be associated with SCD risk in population-based studies is a nonsynonymous single nucleotide polymorphism (SNP) resulting in an amino acid substitution (Gln27Glu, rs1042714) in *ADRB2* gene, which encodes the β 2-adrenergic receptors (β 2AR), an important mediator of the cardiovascular response to sympathetic activation. Sotoodehnia et al. found an increased risk of SCD among an unselected population of elderly individuals homozygous for the Gln27 amino acid polymorphism and validated this finding in a younger population without overt heart disease.¹¹ Subsequently, Tseng et al failed to validate this association in two studies involving individuals with known coronary artery disease (CAD)¹². The discrepant results could be due to small sample sizes of individual studies resulting in either spurious associations or lack of power to detect true associations. Alternatively, associations may differ with variable control for cardiovascular disease.

We sought to explore and attempt to replicate the previously reported association between the Gln27Glu polymorphism and SCD in a large combined sample of SCD cases among individuals of European ancestry assembled from six NIH-funded prospective cohorts utilizing a prospective nested case-control design. We then combined these results with those previously reported among individuals of similar ethnicity in an expanded meta-analysis.

METHODS

Study Populations

Subjects in this nested case-control study were sampled from six prospective cohorts and clinical trials comprising a total of 40,878 men and 67,093 women with stored blood samples. The cohorts included the Physicians' Health Study I and II (PHS I and II), the Nurses' Health Study (NHS), the Health Professionals Follow-up Study (HPFS), the Women's Health Study (WHS), and the Women's Antioxidant Cardiovascular Study (WACS). The PHS I, WHS, and WACS studies were initially randomized trials of vitamin supplementation and/or aspirin. Treatment has ended in these trials, but prospective follow-up is ongoing in PHS I and WHS. The PHS II is an ongoing randomized trial of vitamin supplementation. The NHS and HPFS are observational cohort investigations. Information

regarding medical history, incident disease, and lifestyle changes are assessed either annually or biennially by self-administered questionnaires.

Endpoint Confirmation

The primary endpoint included cases of sudden and/or arrhythmic cardiac death that occurred after return of the blood sample and before April 1, 2007. A total of 540 sudden and/or arrhythmic deaths occurred among participants who donated blood samples at baseline, and 536 of these had DNA samples that passed our quality control standards. Because only 20 cases of SCD occurred among non-European ethnicities, analyses were limited to SCD cases among individuals of European ancestry.

Methods to document the timing and mechanism of cardiovascular deaths were similar across cohorts and have been described previously¹³. Briefly, definite SCDs were defined as death or cardiac arrest occurring within one hour of symptom onset or those with an autopsy consistent with SCD (n= 412, 76.9%). Unwitnessed deaths or deaths occurring during sleep were classified as probable SCDs if the subject was observed to be symptom free in the preceding 24 hours and the circumstances of the death suggest that it could have been sudden (n= 92, 17.2%). Deaths were also classified as arrhythmic or non-arrhythmic based on the definition of Hinkle and Thaler¹⁴. Arrhythmic death was defined as an abrupt spontaneous disappearance of pulse without evidence of prior circulatory impairment or neurologic dysfunction. Deaths which fulfilled the criteria for arrhythmic death, but were preceded by greater than one hour of symptoms (n= 32, 6.0%) were also included in the combined endpoint of sudden and/or arrhythmic cardiac death. Of the total 536 sudden and/or arrhythmic deaths, 68 cases (12.7%) had autopsies performed.

Selection of Controls

For each case, up to three control subjects from the same risk set who were alive at the time of the SCD of the case were selected from the same cohort. Each case was matched on sex, age (± 1 year), ethnicity, smoking status (current, never, past), time and date of blood sampling, fasting status, and presence or absence of cardiovascular disease (CVD), which included a history of myocardial infarction, angina, coronary artery bypass grafting, or stroke at the time of blood draw. Subjects within these cohorts are followed for CVD events on either an annual (WHS, WACS, PHS I and II) or biannual basis (HPFS, NHS). There were an additional 69 cases in whom CVD developed after the blood draw but prior to SCD. For these cases, we selected a second set of three controls that also developed CVD between the time of the blood draw and the SCD of the case to explore how much of the overall association with SCD might be explained by development of non-fatal CVD prior to death.

Genotyping and Quality Control

Genomic DNA was extracted from the buffy coat fraction of centrifuged blood using Qiagen Autopure kits (Valencia, CA) in NHS, HPFS, and WACS and from whole blood in PHS I. In WHS and PHS I, DNA was extracted using the MagNA Pure LC instrument with the MagNA Pure LC DNA isolation kit (Roche Applied Science, Penzberg, Germany). All assays were conducted without knowledge of case status, and samples were labeled by study code only. Matched case-control pairs were handled identically, shipped in the same batch, and assayed in the same analytical run.

The Sequenom platform (San Diego, CA) was used to genotype the common variant in the *ADRB2* gene (rs1042714) previously associated with SCD¹¹. The variant encodes a substitution of glutamine for glutamic acid at position 27 (Gln27Glu). Genotypes for 492 cases passed our quality control thresholds (call rate $\geq 90\%$, Hardy-Weinberg equilibrium $p > 0.01$ in controls), and blinded replicate quality control samples were included and

genotyped with 100% concordance. The genotyping call rate for Gln27Glu was 94.9%. The call rates between cases (95.7%) and controls (94.6%) did not differ significantly ($p=0.31$). No deviation from Hardy Weinberg equilibrium was detected in controls in any of the cohorts.

Statistical analysis

Means or proportions for baseline cardiac risk factors were calculated for cases and controls. The significance of differences in risk factor distributions was tested with the Chi-square statistic for categorical variables and with the Student's t-test for continuous variables. We analyzed the association between the SNP and sudden and/or arrhythmic cardiac death using conditional logistic regression analysis. Based upon the genetic model underlying associations with SCD in prior studies^{11, 12}, odds ratios were estimated for each cohort separately under a recessive model of inheritance for the major allele. Fixed effect meta-analyses were conducted based on the summary conditional logistic regression results for each cohort¹⁵, and PROC MIXED in SAS was used for effect estimation. Tests for homogeneity of the genetic effect across sites were conducted using the Q-statistic¹⁶.

Pooled conditional logistic regression models that directly combined the individual cohort data were used to perform secondary analyses. In secondary analyses, we explored the relation between genotype and SCD according to age, sex, or prevalence of known CVD at the time of the blood draw both through pooled stratified models and by adding cross-product terms between genotype and the exposure of interest into the full pooled multivariable model.

For all of the combined analyses, conditional logistic regression models adjusting for increasing levels of CHD risk factors were performed. The primary model adjusted for age only. Additional models further adjusted for body mass index, history of diabetes, hypertension, hyperlipidemia, and smoking (current, past, never), alcohol intake (<weekly, weekly, daily, 2 or more per day), physical activity (at least once per week), aspirin use (> or = 11 days/month), and family history of myocardial infarction.

Expanded Meta-analysis

A fixed effect meta-analysis using inverse-variance weighting based on the summary regression results in individuals of European ancestry from the Cardiovascular Health Study (CHS)¹¹, Cardiac Arrest Blood Study (CABS)¹¹, UCSF SCD Case Control Study (UCSF)¹², and the 6 prospective cohorts included in the present analysis was performed.

Results from the Heart and Estrogen/Progestin Replacement Study (HERS) trial were not included in the meta-analysis since results were not reported by ethnicity¹². To maintain similarity between the study designs, the pre-specified meta-analysis included the results from UCSF SCD Case Control Study utilizing the control samples without CHD. Standard error was derived from the confidence intervals using the formula $[(\log(\text{upper CI}) - \log(\text{lower CI})) / 3.92]$. The weight was defined as $1 / (\text{SE} * \text{SE})$. All statistical analysis was performed using SAS statistical software (SAS Institute Inc, Cary, NC), Version 9.1.

RESULTS

Study Sample Characteristics

Among Caucasians, 492 cases of sudden and/or arrhythmic cardiac death (179 in women and 313 in men) and 1388 controls matched on age, sex, and prior history of CVD in the six cohorts were successfully genotyped for Gln27Glu. The clinical characteristics recorded at the time of the blood draw for the cases and controls by study cohort are displayed in Table

1. The mean age of the cases was 64.2 years, which varied from 58.8 in WHS to 73.2 in PHS II. Approximately 25% of cases and controls reported a history of CVD prior to the initial blood draw. In pooled analyses, cases were more likely to report a history of diabetes, hypertension, and a higher BMI ($P < 0.05$ for all comparisons). Cases did not significantly differ from controls in any of the other measured cardiac risk factors.

Associations with Sudden/Arrhythmic Cardiac Death

The individual age-adjusted cohort-specific associations for Gln27Glu and sudden/arrhythmic death under a recessive model of inheritance are displayed in Table 2. Allele frequencies among controls were comparable to those reported among Caucasian controls in prior studies.^{11, 12} There was a positive (albeit non-significant) association between individuals homozygous for Gln27 and sudden/arrhythmic death in all cohorts except the WHS. When these odds ratios were combined in meta-analysis adjusted for age, the association approached statistical significance (OR=1.22, 95%CI: 0.98-1.53, $P=0.08$, Q-statistic=1.79; P for heterogeneity = 0.88). After controlling for age, history of hypertension, diabetes, high cholesterol, smoking status, BMI, alcohol, physical activity, aspirin use, and family history of MI, the association between Gln27 homozygosity and sudden/arrhythmic death became marginally significant (OR=1.30, 95%CI: 1.01-1.67, $P=0.046$; Q-statistic=0.53; P for heterogeneity = 0.99) (Table 3). The direction of the association was consistent in sensitivity analyses limited to 410 definite SCDs (OR=1.32, 95%CI: 0.99-1.75) and to 128 SCDs documented to have ventricular fibrillation at the time of the arrest (OR=1.29; 95%CI: 0.60-2.79)

Stratified analysis

In exploratory secondary analyses, we repeated the full pooled multivariable analysis after individually stratifying on the matching variables of age, sex, and history of prior CVD (Table 4). The odds ratios associated with the risk allele were somewhat higher among older individuals, males, and those without a prior history of CVD; but interaction tests in full multivariable models were not significant. Our power to detect such interactions was limited.

Effect Mediation by Interim Development of CVD

There were 69 cases in which CVD developed after the original blood draw, but before reaching the endpoint of sudden/arrhythmic death. In secondary analyses utilizing the alternative set of controls matched on the development of CVD after the blood draw, the association between the Gln27 homozygosity and sudden/arrhythmic death was attenuated and became non-significant in all multivariable models (Last column, Table 3). Among the two sets of controls (178 with CVD, 183 without CVD), homozygosity for Gln27 in *ADRB2* was associated with non-fatal CVD even after controlling for all CVD risk factors included in multivariable model 3 (OR=1.87, 95%CI: 1.15-3.03, $P=0.01$).

Combined Expanded Meta-Analysis

A fixed-effect meta-analysis was used to combine the age-adjusted results obtained in the primary analysis with the unadjusted or age-adjusted analyses reported in 3 separate populations (Table 5). In this combined meta-analysis, Gln27 homozygosity was significantly associated with increased odds of SCD (OR=1.35, 95%CI: 1.15-1.60; $P=0.0003$) (Table 5). When the odds ratios adjusted for cardiovascular risk factors from these 9 studies were combined, the association remained (OR=1.43, 95%CI: 1.20-1.71; $P=0.0001$).

DISCUSSION

This combined analysis of six prospective cohorts adds importantly to the totality of evidence available regarding the association between common variation in *ADRB2* and SCD risk among individuals of European ancestry. After controlling for multiple cardiovascular risk factors, individuals homozygous for Gln27 in *ADRB2* were at a 30% increased risk of sudden/arrhythmic death when compared to individuals with at least one copy of the Glu27 allele. When these results were combined with those from previously published reports, a highly significant association between *ADRB2* genotype and SCD emerged in both the unadjusted (OR=1.35, 95% CI: 1.15-1.60; P=0.0003) and adjusted (OR=1.43, 95% CI: 1.20-1.71; P= 0.0001) meta-analysis. With 885 cases of SCD, this expanded meta-analysis offers the most robust aggregated evidence for a bona fide association between *ADRB2* genotype and SCD to date. Our study also suggests that at least part of this association appears to be mediated by the development of CVD prior to the SCD.

ADRB2 encodes the β_2 -adrenergic receptor, a 7-transmembrane G_s -protein coupled receptor¹⁷. Sympathetic activation mediated by cardiac β_1 - and β_2 -adrenergic receptors (β_1 AR, β_2 AR) plays a major role in triggering and modulating vulnerability to ventricular arrhythmias in diverse disease states^{9, 10, 18}. Stimulation of β_2 ARs on myocardial presynaptic nerve terminals increases cardiac norepinephrine release¹⁹, and β_2 ARs on myocardial cells appear to oppose β_1 AR signaling by switching sequentially from G_s to an inhibitory G protein (G_i) blocking adenylate cyclase.²⁰ The switching results in the production of different cAMP pools in the cardiomyocyte.²¹ β_2 ARs are also located on vascular smooth muscle cells, where activation leads to marked vasodilation. Arterial vasodilator responses have been found to be greater in Glu27 versus Gln27 homozygotes²², and in vitro studies suggest that the Glu27 genotype confers relative resistance to agonist induced receptor down regulation^{17, 23}. How these alterations in β_2 AR function might contribute to SCD risk is not clear, and it is possible that β_2 AR may also influence SCD risk through effects on non-cardiovascular tissues. For example, catecholamine stimulation leads to release of nonesterified fatty acids (NEFA) from adipocytes. The Gln27 allele has been associated with higher levels of circulating NEFA²⁴, which have been linked to an elevated SCD risk in population-based studies²⁵.

Our data suggest that one mechanism through which common variation in *ADRB2* influences SCD risk is through the development of atherosclerosis rather than a direct effect on arrhythmogenesis. When control samples were additionally matched for the development of nonfatal cardiovascular disease prior to SCD, the association between *ADRB2* and SCD was attenuated. Homozygosity for the Gln27 variant was also associated with nonfatal CVD in a subset of matched control samples. However, the associations with CVD and SCD do not appear to be mediated through traditional CHD risk factors. Although homozygosity for the Gln27 polymorphism was not associated with non-sudden cardiac death in the Cardiovascular Health Study¹¹, a prior report from this same study reported an association with both fatal and non-fatal coronary events²⁶. This association was found primarily among patients without clinical cardiovascular disease at baseline. Taken together, these data suggest that part of the observed association with SCD may be mediated through subclinical CHD, which might also explain why an association between *ADRB2* genotype and SCD was not found in the UCSF and HERS studies¹², where controls had established CHD.

There are several strengths of the present study. Strengths include the case-control design nested within large well-characterized prospective cohorts. The combined number of rigorously confirmed sudden and/or arrhythmic cardiac deaths is three-fold that of any cohort in which the Gln27Gly polymorphism had been previously studied. Combining these

data with those previously published in an expanded meta-analysis further strengthened our ability to establish an association. The prospective design also allowed us to match on time at risk, which limits survival bias, and to ascertain cases and controls from the same cohort, which reduces selection bias.

Potential study limitations also require discussion. First, SCD is a difficult phenotype to characterize within populations, and the potential for misclassification always exists. The autopsy rate, although higher than estimated in the general US population²⁷, is quite low in these prospective cohorts. Therefore, we can not rule out other causes of death with certainty. However, the one-hour definition utilized has been documented to have a reasonable sensitivity and specificity for arrhythmic death^{5, 14}, and a sensitivity analysis excluding probable cases, where timing was not known, did not fundamentally alter the results. Second, the low frequency at which SCD occurs in the general population limits the number of SCD cases even in large study samples. Therefore, we needed to combine cases from independent cohorts to achieve adequate power. Third, the study samples were limited to Caucasians; therefore, results cannot be extrapolated to other ethnicities. Also, the selective composition of the cohorts, U.S. health professionals, may limit the generalizability of the findings to other Caucasian populations with differing prevalence of CVD, although other studies in the literature do not have a similar ascertainment. Finally, it is possible that the observed association reflects alternative susceptibility variants in linkage disequilibrium with the Gln27Glu variant.

In conclusion, in this large prospective case-control series, we replicate the association between homozygosity in the Gln27 allele in *ADRB2* and SCD after controlling for multiple cardiovascular and lifestyle risk factors. Further evidence of an association is provided when these results are pooled in meta-analysis with previously published reports. While, the association does not appear to be mediated through traditional risk factors, attenuation on adjustment suggests that it is partly mediated by CVD. Additional investigation is needed to elucidate the biological mechanisms by which this common variant in *ADRB2* predispose to fatal arrhythmias, which may lead to important insights regarding adrenergic mechanisms underlying SCD in the general population.

Acknowledgments

Sources of Funding: Supported by Grant from the National Heart, Lung, and Blood Institute (HL-68070) and a Lerner Young Investigator Award to Dr. Albert, a Doris Duke Charitable Foundation Clinical Scientist Development Award to Dr. Newton-Cheh. The cohort studies were supported by grants: HL-26490, HL-34595, HL-34594, HL-35464, HL-43851, HL-46959, HL-80467 from the National Heart, Lung, and Blood Institute and CA-34944, CA 40360, CA-47988, CA55075, CA-87969, CA 97193 from the National Cancer Institute.

Abbreviations

SNP	Single nucleotide polymorphism
ADRB2	Beta-2 Adrenergic Receptor gene
SCD	Sudden cardiac death
β1AR and β2AR	Beta-1 and Beta-2 adrenergic receptor
OR	Odds ratio
CI	Confidence interval
CAD	coronary artery disease
PHS I and II	Physicians' Health Study I and II

NHS	Nurses' Health Study
HPFS	Health Professionals Follow-up Study
WHS	Women's Health Study
WACS	Women's Antioxidant Cardiovascular Study
CVD	Cardiovascular disease
CHS	Cardiovascular Health Study
CABS	Cardiac Arrest Blood Study
UCSF	UCSF SCD Case Control Study

REFERENCES

1. Zheng ZJ, Croft JB, Giles WH, Mensah GA. Sudden cardiac death in the United States, 1989 to 1998. *Circulation*. 2001; 104:2158–63. [PubMed: 11684624]
2. Lloyd-Jones D, Adams R, Carnethon M, et al. Heart disease and stroke statistics--2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*. 2009; 119:e21–181. [PubMed: 19075105]
3. Gillum RF. Sudden coronary death in the United States: 1980-1985. *Circulation*. 1989; 79:756–65. [PubMed: 2924409]
4. Kannel WB, Wilson PW, D'Agostino RB, Cobb J. Sudden coronary death in women. *Am Heart J*. 1998; 136:205–12. [PubMed: 9704680]
5. Albert CM, Chae CU, Grodstein F, et al. Prospective study of sudden cardiac death among women in the United States. *Circulation*. 2003; 107:2096–101. [PubMed: 12695299]
6. Jouven X, Desnos M, Guerot C, Ducimetiere P. Predicting sudden death in the population: the Paris Prospective Study I. *Circulation*. 1999; 99:1978–83. [PubMed: 10209001]
7. Dekker LR, Bezzina CR, Henriques JP, et al. Familial sudden death is an important risk factor for primary ventricular fibrillation: a case-control study in acute myocardial infarction patients. *Circulation*. 2006; 114:1140–5. [PubMed: 16940195]
8. Kaikkonen KS, Kortelainen ML, Linna E, Huikuri HV. Family history and the risk of sudden cardiac death as a manifestation of an acute coronary event. *Circulation*. 2006; 114:1462–7. [PubMed: 17000909]
9. Podrid PJ, Fuchs T, Candinas R. Role of the sympathetic nervous system in the genesis of ventricular arrhythmia. *Circulation*. 1990; 82:1103–13. [PubMed: 1973640]
10. Barron HV, Lesh MD. Autonomic nervous system and sudden cardiac death. *J Am Coll Cardiol*. 1996; 27:1053–60. [PubMed: 8609321]
11. Sotoodehnia N, Siscovick DS, Vatta M, et al. Beta2-adrenergic receptor genetic variants and risk of sudden cardiac death. *Circulation*. 2006; 113:1842–8. [PubMed: 16618831]
12. Tseng ZH, Aouizerat BE, Pawlikowska L, et al. Common beta-adrenergic receptor polymorphisms are not associated with risk of sudden cardiac death in patients with coronary artery disease. *Heart Rhythm*. 2008; 5:814–21. [PubMed: 18534365]
13. Newton-Cheh C, Cook NR, VanDenburgh M, Rimm EB, Ridker PM, Albert CM. A common variant at 9p21 is associated with sudden and arrhythmic cardiac death. *Circulation*. 2009; 120:2062–8. [PubMed: 19901189]
14. Hinkle LE Jr, Thaler HT. Clinical classification of cardiac deaths. *Circulation*. 1982; 65:457–64. [PubMed: 7055867]
15. Whitehead, A. *Meta-analysis of Controlled Clinical Trials*. Wiley; New York: 2002.
16. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986; 7:177–88. [PubMed: 3802833]

17. Drysdale CM, McGraw DW, Stack CB, et al. Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. *Proc Natl Acad Sci U S A*. 2000; 97:10483–8. [PubMed: 10984540]
18. Altschuld RA, Billman GE. beta(2)-Adrenoceptors and ventricular fibrillation. *Pharmacol Ther*. 2000; 88:1–14. [PubMed: 11033381]
19. Newton GE, Azevedo ER, Parker JD. Inotropic and sympathetic responses to the intracoronary infusion of a beta2-receptor agonist: a human in vivo study. *Circulation*. 1999; 99:2402–7. [PubMed: 10318661]
20. Xiao RP, Cheng H, Zhou YY, Kuschel M, Lakatta EG. Recent advances in cardiac beta(2)-adrenergic signal transduction. *Circ Res*. 1999; 85:1092–100. [PubMed: 10571541]
21. Nikolaev VO, Moshkov A, Lyon AR, et al. Beta2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation. *Science*. 2010; 327:1653–7. [PubMed: 20185685]
22. Cockcroft JR, Gazis AG, Cross DJ, et al. Beta(2)-adrenoceptor polymorphism determines vascular reactivity in humans. *Hypertension*. 2000; 36:371–5. [PubMed: 10988267]
23. Green SA, Turki J, Innis M, Liggett SB. Amino-terminal polymorphisms of the human beta 2-adrenergic receptor impart distinct agonist-promoted regulatory properties. *Biochemistry*. 1994; 33:9414–9. [PubMed: 7915137]
24. Carlsson M, Orho-Melander M, Hedenbro J, Groop LC. Common variants in the beta2-(Gln27Glu) and beta3-(Trp64Arg)--adrenoceptor genes are associated with elevated serum NEFA concentrations and type II diabetes. *Diabetologia*. 2001; 44:629–36. [PubMed: 11380082]
25. Jouven X, Charles MA, Desnos M, Ducimetiere P. Circulating nonesterified fatty acid level as a predictive risk factor for sudden death in the population. *Circulation*. 2001; 104:756–61. [PubMed: 11502698]
26. Heckbert SR, Hindorff LA, Edwards KL, et al. Beta2-adrenergic receptor polymorphisms and risk of incident cardiovascular events in the elderly. *Circulation*. 2003; 107:2021–4. [PubMed: 12682000]
27. Shojania KG, Burton EC, McDonald KM, Goldman L. Changes in rates of autopsy-detected diagnostic errors over time: a systematic review. *Jama*. 2003; 289:2849–56. [PubMed: 12783916]

Figure 1.

Forest plot demonstrating results of the individual studies included in the meta-analysis. Shown below are the age- and sex- adjusted odds ratios (95% CI) limited to Caucasians for SCD among Gln27 homozygotes versus Glu27 carriers for all studies except for Sotoodehnia et al (CABS) studies, where an unadjusted P-value was used. For the Tseng et al (UCSF) study, results are for the comparison with controls with no known coronary disease. The size of the symbol for each point estimate reflects the weight given to the each study in the meta-analysis.

Cohort Specific and Pooled Prevalence of Cardiac Risk Factors at the Time of Blood Draw According to Case and Control Status

Table 1

Cohort	Case/ Control	n	Age (SD) years	Body mass index (SD) kg/m ²	History of prior CVD N (%)	Current smoking N (%)	Diabetes N (%)	Hypertension N (%)	High cholesterol N (%)	Family History of MI N (%)	Aspirin Use N (%)
HPFS	case	109	67.7 (7.7)	26.4 (3.7)	41 (37.6)	10 (9.6)	16 (14.7)	56 (51.4)	57 (52.3)	16 (14.7)	40 (37.0)
HPFS	control	297	67.5 (7.5)	25.7 (3.5)	110 (37.0)	29 (10.0)	22 (7.4)	105 (35.4)	129 (43.4)	41 (13.8)	122 (42.1)
PHS I	case	131	59.7 (8.9)	25.4 (3.1)	4 (3.1)	16 (12.2)	14 (10.7)	61 (46.9)	14 (11.6)	20 (15.3)	64 (48.9)
PHS I	control	381	59.6 (9.0)	24.4 (2.9)	9 (2.4)	46 (12.1)	14 (3.7)	86 (22.8)	48 (13.8)	32 (8.4)	180 (47.2)
PHS II	case	73	73.2 (9.1)	26.0 (4.1)	22 (30.1)	3 (4.1)	13 (17.8)	45 (61.6)	29 (40.3)	13 (17.8)	34 (49.3)
PHS II	control	206	73.1 (8.8)	25.3 (2.8)	62 (30.1)	9 (4.4)	12 (5.8)	103 (50.0)	89 (43.2)	23 (11.2)	109 (53.7)
NHS	case	107	61.1 (6.1)	27.0 (5.4)	31 (29.0)	28 (26.2)	25 (23.4)	66 (61.7)	62 (57.9)	28 (26.2)	35 (32.7)
NHS	control	303	61.2 (5.9)	26.4 (5.1)	88 (29.0)	62 (20.5)	27 (8.9)	138 (45.5)	163 (53.8)	58 (19.1)	65 (21.5)
WACS	case	35	66.2 (7.0)	29.8 (7.2)	25 (71.4)	9 (25.7)	11 (31.4)	28 (80.0)	24 (68.6)	9 (25.7)	17 (48.6)
WACS	control	100	66.1 (6.6)	29.2 (6.1)	72 (72.0)	25 (25.0)	19 (19.0)	74 (74.0)	77 (77.0)	36 (36.0)	48 (48.0)
WHS	case	37	58.8 (8.6)	26.8 (4.5)	1 (2.7)	9 (24.3)	4 (10.8)	20 (54.1)	11 (29.7)	3 (8.1)	16 (43.2)
WHS	control	101	58.4 (8.5)	26.4 (5.1)	1 (1.0)	25 (24.8)	5 (5.0)	34 (33.7)	40 (39.6)	14 (13.9)	59 (58.4)
Total	case	492	64.2 (9.4)	26.5 (4.5)*	124 (25.2)	75 (15.4)	83 (16.9)	276 (56.2)*	197 (41.0)*	89 (18.1)	206 (42.3)
Total	control	1388	64.0 (9.3)	25.8 (4.2)	342 (24.6)	196 (14.2)	99 (7.1)	540 (39.0)	546 (40.3)	204 (14.7)	583 (42.3)

* P-value less than 0.05 for comparison with controls.

Table 2

Primary Analysis: Shown are the individual cohort specific and combined meta-analysis age-adjusted odds ratios (95% CI) according to ADRB2 genotype from conditional logistic regression models under a recessive model of inheritance.

Cohort	Case/ Control	n	Glu/Glu	Glu/Gln	Gln/Gln	OR (95% CI)*	P-Value	Weight
HPFS	case	109	0.20	0.47	0.33	1.46 (0.89-2.41)	0.14	15.33
HPFS	control	297	0.19	0.55	0.27			
PHS I	case	131	0.23	0.40	0.37	1.36 (0.90-2.05)	0.14	22.68
PHS I	control	381	0.20	0.49	0.30			
PHS II	case	73	0.22	0.48	0.30	1.16 (0.64-2.12)	0.63	10.56
PHS II	control	206	0.19	0.54	0.26			
NHS	case	107	0.28	0.37	0.35	1.03 (0.63-1.67)	0.92	16.23
NHS	control	303	0.20	0.46	0.33			
WACS	case	35	0.23	0.40	0.37	1.23 (0.56-2.72)	0.60	6.13
WACS	control	100	0.28	0.39	0.33			
WHS	case	37	0.22	0.49	0.30	0.87 (0.37-2.02)	0.74	5.32
WHS	control	101	0.16	0.51	0.33			
Total	case	492	0.23	0.43	0.34	1.22 (0.98-1.53)	0.08	0.88
Total	control	1388	0.20	0.50	0.30			

P-Heterogeneity

* Odds ratios reflect Gln27 homozygotes versus Glu27 carriers.

Multivariate Analyses: Meta-analysis odds ratios (95% CI) with increasing levels of risk factor adjustment according to ADRB2 genotype

Table 3

Genetic Variant	OR(95% CI) for All SCD (Primary Analysis)	P- value	OR(95% CI) for Definite SCD (Sensitivity analysis) *	P- value	OR(95% CI) Alternate Controls matched for Interim CVD ‡	P -value
Multivariable Model 1 †	1.24 (0.98-1.58)	0.077	1.29 (0.99-1.67)	0.059	1.16 (0.92-1.47)	0.22
Multivariable Model 2 §	1.28 (0.99-1.65)	0.055	1.30 (0.99-1.73)	0.064	1.18 (0.92-1.51)	0.20
Multivariate Model 3 ¶	1.30 (1.01-1.67)	0.046	1.32 (0.99-1.75)	0.056	1.19 (0.92-1.52)	0.19

* **Sensitivity Analysis:** Utilizing only cases of definite sudden cardiac death (N=410) defined as death within one-hour of the onset of symptoms or autopsy consistent with SCD (i.e. acute coronary thrombosis or severe coronary artery disease without myocardial necrosis or other pathologic findings to explain death)

† **Secondary Analysis:** Utilizing an alternative set of controls who developed CVD after the blood draw for the 69 cases who developed CVD after the blood draw.

‡ **Multivariable Model 1:** Controlled simultaneously for age, smoking status (current, past, never), BMI (continuous), history of diabetes, hypertension, and high cholesterol

§ **Multivariable Model 2:** Controlled for variables listed above in Model 1 and alcohol intake (<weekly, weekly, daily, 2 or more per day); physical activity (at least once per week) and aspirin (> or = 11 days/month).

¶ **Multivariable Model 3:** Controlled for variables listed above in Model 2 and family history of myocardial infarction

Secondary Stratified Analyses: Shown are the multivariable* odds ratios (95%CI) for sudden cardiac death according to ADRB2 variant using a recessive model of inheritance from pooled conditional regression models with stratification on age, sex, or history of CVD prior to blood draw.

Table 4

Cardiac Risk Factor	Cases (n)	Controls (n)	OR (95%CI)*	P-value (Subgroup)	P-value (Interaction)
Age					
<Median	244	689	1.06 (0.75 - 1.51)	0.74	0.15
≥Median	248	699	1.52 (1.09 – 2.12)	0.013	
Sex					
Male	313	884	1.35 (0.99 - 1.83)	0.05	0.35
Female	179	504	1.09 (0.73 – 1.62)	0.67	
History of CVD					
Yes	124	342	1.04 (0.65 – 1.66)	0.88	0.44
No	368	1046	1.31 (0.99 – 1.73)	0.06	

* Multivariable models controlled simultaneously for age, smoking status (current, past, never), BMI (continuous), history of diabetes, hypertension, and high cholesterol, family history of myocardial infarction, alcohol intake (<weekly, weekly, daily, 2 or more per day); physical activity (at least once per week) and aspirin (> or = 11d/month).